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Original research paper

Urban parks biowaste as a sustainable source of new antidiabetics

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ABSTRACT

Biowaste produced in urban parks is composed of large masses of organic matter that is only occasionally used economically. In this work, extracts of six plants widely distributed in urban parks in Central Europe (*Achillea millefolium*, *Cichorium intybus*, *Malva sylvestris*, *Medicago sativa*, *Plantago lanceolata*, and *Trifolium pratense*), prepared using 10 % and 50 % ethanol, were screened for their antidiabetic and related properties. HPLC and UV-VIS analysis revealed presence of caffeic acid, quercetin, luteolin, and apigenin derivatives. The extracts were active in DPPH antiradical, β-carotene-linoleic acid, ORAC, and reducing power assay. They inhibited lipoxygenase, collagenase, as well as heat-

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induced ovalbumin coagulation. They were also able to hinder carbohydrate degradation. For example, IC_{50} of anti-α-amylase activity of 10 % and 50 % ethanol extract of *M. sativa* extracts (204.10 ± 2.11 μg mL^{-1} and 78.27 ± 0.99 μg mL^{-1}, respectively) did not statistically differ from the activity of the positive control, acarbose (284.74 ± 3.81 μg mL^{-1}). Similar results were observed for their anti-α-glucosidase activity. In most assays, use of 50 % ethanol was shown to be better suited for extraction of active metabolites. The results indicate that the biowaste obtained from urban parks represents a potential source of plant material for the preparation of high-value antidiabetic products.

Keywords: antidiabetic, antihyperglycemic, anti-inflammatory, HPLC, natural phenols, waste management

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INTRODUCTION

Type 2 diabetes (T2D) is a condition frequently associated with poor dietary habits and a lack of physical activity. It is estimated that T2D impacts over 30–40 % of individuals aged 65 and older (1). This disorder is characterized by insulin resistance that leads to elevated blood sugar levels, inflammation (2), and overproduction of reactive oxygen species (ROS), resulting in a persistent state of oxidative stress (3). Despite advancements in treatment strategies for T2D, antidiabetic medications may have serious shortcomings, including the risks of hypoglycemic episodes and complications affecting the liver and kidneys. The use of medicinal plants or plant-based therapies is considered to

be a cost-effective approach used globally for the prevention, amelioration of consequences, and, to some extent, even therapy of T2D. Numerous studies suggest that medicinal plants can influence carbohydrate and lipid metabolism, reduce hyperglycemia, dyslipidemia, and insulin resistance, as well as mitigate oxidative stress and inflammatory responses (4, 5). Medicinal plants contain various secondary metabolites that exhibit antidiabetic properties, and many contemporary pharmaceuticals are derived from phytoconstituents of traditional medicinal flora. For instance, the biguanide antihyperglycemic medication metformin has its origins in the traditional use of *Galega officinalis* L. for diabetes treatment (6). For all this reasons, the World Health Organization (WHO) endorses the incorporation of medicinal plants into dietary practices for the managing of T2D (6).

Among secondary metabolites that influence biological targets and provide therapeutic benefits in the management of T2D, polyphenols occupy a special place. They demonstrate their antidiabetic effects *via* multiple mechanisms, such as antioxidant activity and inhibition of digestive enzymes (7). Numerous studies have shown that the use of complex mixtures of phenolics and other plant metabolites, such as those present in plant extracts, could result in synergistic action of active principles and, consequently in their increased efficiency (8). However, due to their unique chemical characteristics, phytochemicals may display different solubilities in different solvents. Consequently, the choice of solvent can strongly affect the composition and, as a result, biological activity of the prepared extracts (9).

Urban green spaces, particularly urban parks, constitute a vital element of contemporary cities. These areas are characterized by well-maintained vegetation and serve as crucial reservoirs of biodiversity within urban environments. Their landscape, primarily consisting of grasslands interspersed with

occasional trees, bears a strong resemblance to savanna-type ecosystems (10). The biowaste generated in these parks arises as a consequence of the natural growth of plants whose maintenance produce substantial amounts of organic material (11). Despite the significant volume of this biomass, its economic utilization is rather limited, primarily restricted to composting, production or energy generation in biogas facilities or incinerators (12). However, a significant portion of the plants growing in such areas comprise medicinal and edible plants. Therefore, there is a possibility to use this biowaste for the creation of high-value products, including dietary supplements (11), thus lowering the production costs and enhancing its sustainability. A recent study have shown that plants from urban parks may serve as a valuable source of bioactive phenolics suitable for cosmetic applications (13). However, there is still a notable deficiency in research regarding the application of biowaste from urban green spaces in various industrial sectors.

Having in mind abundance of medicinal plants in urban parks biowaste, this work aimed to investigate if this resource can be used for production of high-value antidiabetic products. Six plant species identified as common biowaste in urban parks with potential anti T2D activity, *Achillea millefolium* L., *Cichorium intybus* L. (Asteraceae), *Medicago sativa* L., *Trifolium pratense* L. (Fabaceae), *Malva sylvestris* L. (Malvaceae), and *Plantago lanceolata* L. (Plantaginaceae) were selected for the study. Their phenolic profiles, antioxidant properties, anti-inflammatory, and antihyperglycemic activities were evaluated and compared. To encompass a broader range of metabolites and identify the most suitable solvent for the potential development of medicines and food supplements derived from these plants, extractions were conducted using two solvents with differing polarities: 10 % and 50 % ethanol (*V/V*).

EXPERIMENTAL

Plant materials and chemicals

Flowering aerial parts of selected plants (*A. millefolium*, *C. intybus*, *M. sylvestris*, *M. sativa*, *P. lanceolata*, and *T. pratense*) were collected in the recreational area in the city of Zagreb (lake Jarun, 45°48′N 15°90′E) in June 2019. The identity was confirmed by the authors. Standards for HPLC and spectrophotometric determinations were purchased from Sigma-Aldrich (USA). Soybean lipoxygenase (LOX) was a product from TCI chemicals (Japan), while the other enzymes were purchased from Sigma-Aldrich. The purity of all the standards was ≥ 98.5 %. Other reagents and chemicals were of analytical grade. Extraction was performed using Bandelin SONOREX® Digital 10 P DK 156 BP ultrasonic bath (Germany). Agilent 1200 series HPLC instrument equipped with an autosampler, DAD detector, and a Zorbax Eclipse XDB-C18 (5 μm, 250 mm × 4.6 mm) column and guard column (Agilent Technologies, USA) was used for the determination of the selected secondary metabolites. A microplate reader (FLUOstar Omega, BMG Labtech, Germany) was used for UV-VIS spectroscopic measurements.

Preparation of the extracts

Prior to the extraction, plant material was grinded and passed through a sieve of 850 μm mesh size. Thereafter, to the 3 g of powdered plant material placed in a 100 mL Erlenmeyer flask, 30 mL of the appropriate solvent (10 % or 50 % ethanol) was added. The flask was then placed in the ultrasonication bath. The extraction was performed at 25 °C for 20 min at 72 W power. Upon the extraction, the mixture was filtered, and the remaining material at filter paper was washed 2 times with 30 mL of the same solvent. The extracts were combined in a round-bottom flasks and evaporated under reduced pressure. After drying in a desiccator for 24 h over anhydrous silica gel, dry

extracts were stored at +4 °C in the dark until use. The extracts' names were composed using two parts, first being the abbreviation of the plant's name: *A. millefolium* (AM), *C. intybus* (CI), *Malva sylvestris* (MSy), *Medicago sativa* (MS), *P. lanceolata* (PL), and *T. pratense* (TPr), followed by the second part consisting of the number (10 or 50) representing the percentage of ethanol used for the extraction (*e.g.* MSy10).

Spectrophotometric determination of phenolic compounds

Total phenols (TP), content was determined using Folin-Ciocalteau reagent (14), while determination of total flavonoids (TF) was performed in reaction with aluminum chloride (15). Total hydroxycinnamic acids (TCA) content was determined using the reaction with Arnow's reagent (16). Content of TP and TCA was expressed as μg of caffeic acid equivalents (CAE), while the content of TF was expressed as quercetin equivalents (QE) in g of dry mass (DM) of the extract.

HPLC analysis of phenolic constituents

Methanolic solutions of the extracts (2 mg mL^{-1}) and the phenolic standards (0.2 mg mL^{-1} in methanol) were filtered through a 0.45 μm PTFE syringe filter, and subjected to HPLC chromatographic separation at a temperature of 40 °C and flow of 1.2 mL min^{-1}. The solvents A and B were 2 % formic acid (*V*/*V*) and acetonitrile applied as follows 0–23 min 0–20 % B, 23–27 min 20–22.5 % B, 27–28 min 22.5–24.4 % B, 28–30 min 24.4–26.2 % B, 30–50 min 26.2–30 % B, 50–60 min 30–100 % B, 60–61 min 100–0 % B, 61–66 min 0 % B. To construct a calibration curve, varying volumes of standard solutions were injected using an autosampler. The peak assignment and identification was based on comparison of retention times of peaks in sample chromatogram and UV spectra (200–500 nm) with those of the standards. Cafeic acid (CA) and its derivatives,

chlorogenic acid (ChA), neochlorogenic acid (NcA), rosmarinic acid (RA), and verbascoside (V) were used as standards for hydroxycinnamic derivatives. Furthermore, apigenin (A), luteolin (L), and quercetin dihydrate (Q), as well as their derivatives apigenin-7-*O*-glucoside (A7G), luteolin-7-*O*-glucoside (L7G), hyperosid (H), isoquercetin (IQ), and quercitrin dihydrate (Qcit) were used flavonoid standards. Calibration curve parameters are reported in Table I.

Free radical scavenging activity

Radical scavenging activity (RSA) was evaluated (17) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Solution of DPPH (70 μL, 0.21 mg mL^{-1} in methanol) was added to 130 μL of extract solution (sample). After 30 min the absorbance was read at 545 nm. RSA was calculated according to the Equation *1*:

$$\text{RSA}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \qquad (1)$$

where A_{control} is the absorbance of the negative control (solution that contains methanol instead of extract) and A_{sample} is the absorbance of the DPPH solution containing extract. Butylated hydroxyanisole (BHA) was used as the standard radical scavenger.

Antioxidant activity in β-carotene-linoleic acid assay

Antioxidant activity in *β*-carotene-linoleic acid assay (ACLA) was evaluated using modified literature procedure (18). Emulsion (200 μL) containing *β*-carotene (6.7 μg mL^{-1}), linoleic acid (0.7 mg mL^{-1}), and Tween 40 (6.7 mg mL^{-1}) was added either to water (50 μL) (control) or to the solutions of the extracts in methanol (50 μL). The reaction mixtures were subjected to incubation at 50 °C. ACLA was calculated based on the absorbance 459 nm recorded each 5 min for 60 min using the Equation *2*:

$$ACLA\ (\%) = \frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \times 100, \quad (2)$$

where R_{control} and R_{sample} are the average reaction rates of *β*-carotene bleaching for the water control and the antioxidant, respectively, calculated according to the pseudo-first order kinetics. BHA was used as the standard antioxidant.

Reducing power assay

The reducing power of extracts (RP) was determined according to the previously used method (19) modified for microtiter plate. The extract solution (80 µL), phosphate buffer (0.2 mol L^{-1}, pH = 6.6, 100 µL), and potassium ferricyanide (1 % *m/V*, 100 µL) were incubated for 20 min at 50 °C. Thereupon, trichloroacetic acid (10 % *m/V*, 100 µL) was added. An aliquot of 125 µL was transferred to a new plate. After two-fold serial dilution ferric chloride (25 µL, 0.1 %) solution was added. The absorbance was read spectrophotometrically at 700 nm. Ascorbic acid was used as the positive control.

Oxygen radical absorbance capacity

For the oxygen radical absorbance capacity (ORAC) assay (20), 25 µL of the extract solution or phosphate buffer (75 mmol L^{-1}, pH 7.4) (blank), and 150 µL of fluorescein (1 µmol L^{-1}) were preincubated 37 °C. After 10 min, 25 µL of 125 mmol L^{-1} 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added and the fluorescence was measured kinetically at 37 °C every 150 seconds for 60 min. The excitation and emission wavelengths were 485 nm and 528 nm, respectively. The ORAC activity of a sample was calculated by subtracting the area under the blank curve from the area under the sample curve to obtain the net area under the curve. Using Trolox of known concentration, a standard curve was generated and the ORAC activity of the samples was calculated as Trolox equivalents.

264 *Table I. Calibration curve parameters for selected phenolic standards*

Phenolic standard	λ (nm)	Equation	R^2	*LOD* (μg)	*LOQ* (μg)
Apigenin	365	$y = 1545.95x + 4.48$	0.9999	0.002	0.006
Apigenin-7-*O*-glucoside	365	$y = 4708.27x + 184.78$	0.9998	0.084	0.279
Caffeic acid	320	$y = 4004.80x + 28.93$	0.9999	0.016	0.053
Chlorogenic acid	320	$y = 2020.89x – 17.88$	0.9999	0.033	0.110
Hyperoside	365	$y = 1567.21x + 9.76$	0.9999	0.007	0.024
Isoquercetin	365	$y = 1286.94x + 14.32$	0.9999	0.004	0.014
Luteolin	365	$y = 2504.96x + 9.67$	0.9999	0.004	0.014
Luteolin-7-*O*-glucoside	365	$y = 1333.63x + 9.73$	0.9999	0.008	0.028
Neochlorogenic acid	320	$y = 1379.16x + 35.41$	0.9999	0.021	0.070
Quercetin dihydrate	365	$y = 1872.38x –1.96$	0.9999	0.002	0.006
Quercitrin dihydrate	365	$y = 1055.99x + 4.60$	0.9999	0.018	0.058
Rosmarinic acid	320	$y = 1945.38x + 16.34$	0.9999	0.007	0.025
Verbascoside	320	$y = 346.84x + 8.98$	0.9999	0.037	0.123

265 *LOD* – level of detection, *LOQ* – level of quantification, *y* – area under curve (mAU×s), *x* – amount of the standard (μg).

Lipoxygenase inhibitory activity

For determination of lipoxygenase inhibitory activity (LOXI) (21), 50 μL of the extract solution was mixed with 150 μL phosphate buffer (pH 8, 100 μmol L^{-1}) and 20 μL of soybean LOX solution (1 mg mL^{-1}). Thereupon, linoleic acid (30 μL) was added and the mixture incubated at 25 °C. After 10 min the absorbance was determined at 234 nm. The activity was calculated as described in Equation *3*:

$$LOX\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \quad (3)$$

where A_{control} is the absorbance of the negative control (reaction mixture prepared with water instead of the extract) and A_{sample} is the absorbance of the respective extract. Nordihydroguaiaretic acid (NDGA) was used as positive control.

Ovalbumin coagulation assay

Heat-induced ovalbumin coagulation assay (OVA) (22) was used for determination of anti-inflammatory activity. Ovalbumin solution (0.4 mL of 50 % fresh hen's albumen) was mixed with phosphate buffered saline (2.8 mL, pH 6.4) and the extract solutions (2 mL). The mixtures were first incubated at 37 °C for 15 min and then heated at 70 °C for 5 min. After an additional 10 min at room temperature, their absorbance was recorded at 660 nm. OVA was calculated using the Equation *4*:

$$\text{OVA}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \quad (4)$$

where A_{control} is the absorbance of the negative control (reaction mixture prepared with water instead of the extract) and A_{sample} is the absorbance of the respective extract. Diclofenac sodium was used as the standard coagulation inhibitor.

Collagenase inhibitory activity

Collagenase inhibitory activity assay (COLL) was performed according to Zhanga *et al.* (23) with some modifications. To 40 µL of the extract solution in Tris-HCl buffer (0.1 mol L^{-1}, pH 7.5, 5 mmol L^{-1} $CaCl_2$, and 1 µmol L^{-1} $ZnCl_2$), 20 µL of collagenase dissolved in the same buffer (1 mg mL^{-1}) was added. After 5 min at room temperature, gelatin dissolved in the same buffer (30 µL, 3.5 mg mL^{-1}) was added and the mixture was incubated at 37 °C. After 40 min, stop reagent, containing 12 % (*m/V*) PEG 6000 and 25 mmol L^{-1} EDTA was added, followed by 90 µL of the ninhydrin reagent. The reaction mixture was incubated for 15 min at 80 °C and the absorbance measured at 570 nm. The activity was calculated by using the Equation *5*:

$$\text{COLL}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \qquad (5)$$

where A_{control} is the absorbance of the negative control (reaction mixture prepared with water instead of the extract) and A_{sample} is the absorbance of the respective extract. Gallic acid was used as the positive control.

α-Amylase inhibition assay

For *α*-amylase inhibition assay (aAM) (24) the extract solution (0.5 mL) and phosphate buffer (0.5 mL, pH 6.9, 20 mmol L^{-1}), containing *α*-amylase from porcine pancreas (0.8 IU mL^{-1}), were mixed and incubated at 25 °C. After 10 min soluble starch (0.5 mL, 0.5 % solution in the same buffer) was added and the reaction mixture incubated at 25 °C for 10 min. The reaction was stopped with 1 mL of 96 mmol L^{-1} 3.5-dinitrosalicylic acid, and the reaction mixture incubated in a boiling water bath for 5 min. After cooling to room temperature, the reaction mixtures were diluted with 10 mL distilled water and the absorbance was measured at 540 nm. The activity was calculated as shown in Equation *6*:

$$\text{aAM}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \quad (6)$$

where A_{control} is the absorbance of the negative control (reaction mixture prepared with water instead of the extract) and A_{sample} is the absorbance of the reaction mixture containing extracts. Acarbose was used as the positive control.

α-Glucosidase inhibition assay

For inhibition of *α*-glucosidase determination (aGA) (25) extract solution (20 μL) was incubated with 50 μL of *α*-glucosidase from *Saccharomyces cerevisiae* (0.2 U mL^{-1} dissolved in 0.1 mol L^{-1} phosphate buffer, pH 6.8) for 10 min at 37 °C. Substrate (50 μL of 1 mmol L^{-1} *p*-nitrophenyl- *α*-D-glucopyranoside prepared in same buffer) was added to the reaction mixture and the absorbance at 405 nm was recorded after 5 min. The activity was calculated according to the Equation *7*:

$$\text{aGA}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100, \quad (7)$$

where A_{control} is the absorbance of the negative control (reaction mixture prepared with water instead of the extract) and A_{sample} is the absorbance of the reaction mixture containing extracts. Acarbose was used as the positive control.

Statistical analysis

The measurements were performed in triplicate and the results presented as mean ± standard deviation. Scavenging activity for DPPH free radical, antioxidant activity in *β*-carotene-linoleic acid assay, lipoxygenase inhibitory activity, activity in the ovalbumin coagulation assay, collagenase inhibitory activity, *α*-amylase inhibitory activity, and *α*-glucosidase inhibitory activity were calculated as the concentration of the extract that displays 50 % of the desired activity (IC_{50}). Activity in the RP assay was calculated as the concentration that increases the absorbance of reaction mixture

to the value of 0.5 at 700 nm ($EC_{0.5}$). For the ORAC assay a standard curve was generated using Trolox of known concentrations. The activity in the ORAC assay was calculated as Trolox equivalents per gram of plant material (μmol TE g^{-1} DM), while IC_{50} and $EC_{0.5}$ values were expressed as mass concentration of plant material (μg DM mL^{-1}). For each assay, four to seven different concentrations (depending on the assay) were prepared using 2-fold dilution and used for determination of the respective activity. The values were calculated using regression analysis. The extracts that did not display observable inhibitory activity in the performed assay, as well the extracts for whom the calculated IC_{50} value in the respective assay was higher than 5000 μg DM mL^{-1}, were considered inactive and excluded from the subsequent statistical analysis. Statistical comparisons were performed using one-way ANOVA followed by either Dunnett (comparisons of the individual extracts with the controls) or Tukey (for comparisons between the extracts) post-hoc tests. The differences between ethanolic and aqueous extracts were investigated using paired *t*-test. Unless otherwise noted, p values < 0.05 were considered statistically significant. For overall comparison of the activity among the extracts, the activity in each assay was normalized. First, the reciprocal value of each IC_{50} value was calculated, while RP $EC_{0.5}$ and TE ORAC values were used unchanged. The normalized values were calculated as the percentage of the activity achieved by the most active extracts. The normalized values were then summed for each group of assays to give estimation of the overall most active extracts.

RESULTS AND DISCUSSION

Phenolic content of the extracts

While some plants are cultivated specifically for the needs of the food supplement industry, many are still collected in nature. Each practice has its own drawbacks as the former

generates a significant amount of waste, while the latter has the potential to damage natural resources and disrupt sensitive ecological balance. On the other hand, utilizing existing biowaste to obtain high-value products, such as food supplements, may be a sustainable approach for their production. In this regard, use of biowaste that remains as the residue after mowing of urban parks, may be a promising approach as this practice may generate a large amount of biomass mostly discarded as waste (11).

In this study, six plants, representing the common waste remaining after mowing urban parks in continental Europe, were selected for screening. Aerial parts of some of the plants investigated in this study are commonly used as traditional antidiabetics in Croatia (AM and CI) (26), or other countries (MS, TPr) (8, 27), while the specific antidiabetic ethnopharmacological use of the leaves, flowers and the stems of the others (MSy, PL) has not been recorded so far. A common feature of the plants used in this study is that they are often undervalued and underutilized, yet have the means of potentially creating novel, value-added, food and supplements. Previous studies identified them as rich sources of natural phenols including hydroxycinnamic acid derivatives and natural flavonoids (28). This is of particular interest as natural phenols deservedly keep their place in the spotlight of scientific research of diabetes. They exhibit a variety of beneficial effects related to the prevention of T2D and associated complications. Phenolics are recognized for their antioxidant and anti-inflammatory properties that contribute to the reduction of oxidative stress resulting from elevated glucose levels in T2D. They also have the potential to inhibit digestive enzymes that influence glucose levels in the blood, such as α-glucosidases and α-amylase. A significant number of their effects is, directly or indirectly, linked to the protection of pancreatic β-cells against glucose-induced toxicity, stimulation of the insulin production, and

reduction of insulin resistance (7). All this may result in the prevention of secondary T2D complications as it has been repeatedly shown that consumption of phenolic-rich foods contributes to protection of the eye, kidney, and liver the organs most sensitive secondary complications in T2D (4).

In this work, TP content, as well as the content of the two widely distributed groups of phenols, flavonoids (TF), and hydroxycinnamic acids (TCA), was investigated and presented in Table II. In order to estimate the effects and influence of individual flavonoids and hydroxycinnamic acids on the potential antidiabetic effects of the extracts, HPLC analysis was used and the results presented in Table III. While the extracts were quite rich in the phenolic compounds, significant differences were present among extracts (Table II). Richest in TP was CI50 (274.81 ± 7.31mg CAE g^{-1} DM), closely followed by MS50. TP content was the lowest in the MSy based extracts, MSy10, and MSy50 (102.34 ± 3.14 mg CAE g^{-1} DM and 128.91 ± 8.39 mg CAE g^{-1} DM, respectively).

Hydroxycinnamic acids are among the most ubiquitous phenolic compounds in nature. They largely belong to the group of phenolic acids, and similar to other phenolics, they have the ability to influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion, stimulation of insulin secretion from the pancreatic β-cells, modulation of glucose release and output from liver, as well as by the activation of glucose uptake in insulin-sensitive tissues (29). The results presented in Table II show that the prepared extracts contained considerable amounts of TCA. They constituted up to 70 % of TP in PL extracts, and about 50 % in AM extracts. Among the analyzed extracts, TCA content was highest in the extracts prepared from *P. lanceolata*, PL50 (165.4 ± 1.45 mg CAE g^{-1} DM) and PL10 (138.14 ± 2.38 mg CAE g^{-1} DM). Again, MSy10 and MSy50 were the extracts with the lowest amount of TCA

(19.74 ± 1.79 mg CAE g^{-1} DM and 21.32 ± 1.91 mg CAE g^{-1} DM, respectively).

Among the hydroxycinnamic acids, the most widespread and often analyzed for their antidiabetic activities are CA derivatives. Pharmacological studies have shown that dietary supplementation with CA lowers blood glucose and enhances insulin levels in diabetic mice. In addition, it reduces the levels of plasma HbA1c, urinary glycated albumin, renal carboxymethyl lysine, pentosidine, sorbitol, and fructose levels, as well as significantly diminishes renal activity of aldose reductase and sorbitol dehydrogenase (29). As shown in Table III, CA was found in the extracts prepared from AM, PL and TPr, however in relatively small quantities (up to 0.205 mg g^{-1} DM in AM-10).

Phenolic compounds in plant materials typically exist in the form of glycosides or esters. Nevertheless, the aglycon type is the primary factor that influences the pharmacological effects of flavonoids, phenolic acids, and their respective preparations (30). Therefore, the presence of some common CA derivatives was also assessed. First among them was NcA, a phenolic acid with a significant antidiabetic potential. It is the main compound of mulberry leaf extract, a well-known antidiabetic remedy in many countries, including Croatia (31). Recent studies have shown that both, mulberry leaf extract and NcA, were able to ameliorate glucolipotoxicity-induced diabetic nephropathy in high-fat diet-fed diabetic mice (32). In this work, NcA was present in the same extracts as CA, albeit in similarly low quantities (Table III). The richest among them was PL50, containing 1.510 mg g^{-1} DM of this phenolic acid On the other hand, RA, a CA derivative similarly beneficial in the amelioration of diabetic neuropathy in rats (29), was present just in the extracts prepared from AM, mostly in AM50 (7.722 mg g^{-1} DM).

492 *Table II. Content of phenolic compounds and antioxidant activity of the extracts*

Extract	TP (mg CAE g^{-1} DM)	TCA (mg CAE g^{-1} DM)	TF (mg QE g^{-1} DM)	RSA IC_{50} (µg g^{-1} DM)	ACLA IC_{50} (µg g^{-1} DM)	RP $EC_{0.5}$ (µg g^{-1} DM)	ORAC (µmol TE g^{-1} DM)
AM10	$154.86 \pm 6.25^{f,g}$	68.03 ± 2.36^{g}	$16.77 \pm 0.68^{f,g}$	$75.73 \pm 1.27^{d,x}$	$39.38 \pm 0.88^{f,x}$	$0.32 \pm 0.04^{d,x}$	1943.55 ± 229.93^{h}
AM50	208.38 ± 11.74^{d}	108.57 ± 0.27^{d}	45.49 ± 0.42^{b}	$40.88 \pm 2.89^{h,i,x}$	$37.26 \pm 0.46^{f,x}$	$0.20 \pm 0.02^{e,f,x}$	$4785.37 \pm 280.08^{b,c}$
CI10	$217.84 \pm 1.93^{c,d}$	85.07 ± 1.88^{e}	58.8 ± 2.87^{a}	$66.93 \pm 0.76^{e,x}$	$57.51 \pm 1.64^{a,x}$	$0.18 \pm 0.01^{e,f,g,h,x}$	$3503.36 \pm 293.10^{e,f}$
CI50	274.81 ± 7.31^{a}	119.95 ± 4.00^{c}	61.09 ± 2.21^{a}	$32.73 \pm 2.22^{i,j,x}$	$39.44 \pm 0.47^{f,x}$	$0.13 \pm 0.01^{g,h,y}$	5368.23 ± 412.84^{b}
MS10	149.57 ± 1.88^{g}	21.82 ± 0.85^{j}	$19.63 \pm 0.40^{e,f}$	$69.68 \pm 0.28^{d,e,x}$	$31.54 \pm 2.80^{g,x}$	$0.20 \pm 0.01^{e,f,g,x}$	$3968.58 \pm 235.63^{c,d,e}$
MS50	268.97 ± 6.08^{a}	40.29 ± 1.52^{h}	29.27 ± 0.89^{c}	$26.94 \pm 0.46^{j,x}$	$40.64 \pm 0.56^{e,f,x}$	$0.12 \pm 0.02^{h,y}$	$4726.89 \pm 307.99^{b,c}$
MSy10	102.34 ± 3.14^{i}	19.74 ± 1.79^{j}	9.98 ± 0.14^{i}	$229.74 \pm 6.91^{a,x}$	$52.77 \pm 0.37^{b,x}$	$0.39 \pm 0.05^{c,x}$	$3044.79 \pm 285.36^{f,g}$
MSy50	128.91 ± 8.39^{h}	21.32 ± 1.91^{j}	$15.17 \pm 0.32^{g,h}$	$173.6 \pm 2.99^{b,x}$	$48.46 \pm 2.94^{c,d,x}$	$0.44 \pm 0.02^{b,c,x}$	$4659.76 \pm 269.22^{b,c}$

PL10	200.77 ± 2.71^{d}	138.14 ± 2.38^{b}	17.86 ± 0.17^{f}	43.00 ± 3.30^{g,h,x}	39.27 ± 0.83^{f,x}	0.19 ± 0.01^{e,f,g,h,x}	2188.12 ± 320.92^{g,h}
PL50	234.41 ± 7.59^{b,c}	165.4 ± 1.45^{a}	23.7 ± 0.80^{d}	41.33 ± 1.42^{h,x}	40.31 ± 0.39^{f,x}	0.15 ± 0.01^{f,g,h,y}	5434.69 ± 170.57^{b}
TPr10	129.47 ± 6.66^{h}	32.07 ± 0.98^{i}	14.31 ± 1.02^{h}	109.28 ± 3.46^{c,x}	52.34 ± 1.32^{b,c,x}	0.52 ± 0.03^{a,x}	3779.84 ± 396.76^{d,e,f}
TPr50	176.26 ± 6.63^{e}	75.84 ± 5.72^{f}	29.4 ± 0.52^{c}	50.86 ± 1.96^{f,g,x}	46.05 ± 0.86^{d,x}	0.22 ± 0.01^{e,x}	7354.71 ± 365.39^{a}
Standard				$^{(1)}$ 9.58 ± 0.38^{y}	$^{(1)}$ 2.03 ± 0.05^{y}	$^{(2)}$ 0.12 ± 0.03^{y}	

TP – total phenols, CAE – caffeic acid equivalents, DM – dry mass of the extract, TCA – total hydroxycinnamic acids, TF – total flavonoids, QE – quercetin equivalents, RSA – scavenging activity for 2,2-diphenyl-1-picrylhydrazyl free radical, IC_{50} – concentration that produces 50 % of the inhibition in the assay, ACLA – antioxidant activity in β-carotene-linoleic acid assay, RP – reducing power, $EC_{0.5}$ – concentration used in the assay that produces the absorption of 0.5, ORAC – oxygen radical absorbance capacity, TE – Trolox equivalents; The extracts' names abbreviations are explained in the Experimental section “Preparation of the extracts”.

Standards: $^{(1)}$ = Butylated hydroxyanisole, $^{(2)}$ = Ascorbic acid.

$^{a\text{-}j}$ = Differences between the extracts within a column (extracts not connected with the same capital letter are statistically different, Tukey post-test, $p < 0.05$); ^{x,y} = differences with the positive control within a column (extracts not connected with the same capital letter are statistically different, Dunnet's post-test, $p < 0.05$). Values are average of 3 replications ± SD.

Conversely, ChA was by far the most widespread CA derivative, being present in all the analyzed extracts in relatively high quantities. This finding is not unexpected because ChA is one of the most abundant phenolic compounds in nature. It is widely considered that numerous benefits of coffee consumption, including lower risk of T2D, are related to its ChA content. T2D-related benefits of ChA include attenuation of the intestinal absorption of glucose, regulation of glucose metabolism via the activation of AMP-activated protein kinase, and improvement of insulin sensitivity through an adiponectin receptor-mediated signaling pathway (29). AM extracts were especially rich in ChA, especially AM-50 with the content of 23.332 mg g^{-1} DM.

Phenylpropanoid glycoside verbascoside (acteoside) is a CA derivative that doesn't belong to the group of phenolic acids. It is present in various plants, most notably in olive fruit and leaf (*Olea europea* L, Oleaceae) where it is considered to largely contribute to the benefits of their consumption. PL is also a plant that is known to contain large amounts of this glycoside (33). Accordingly, it was present in rather high quantities in the extracts prepared from PL, especially in PL50 (453.7 mg g^{-1} DM) (Table III). The amount of verbascoside in PL extracts seemingly outweighed the TP content (Table II). The apparent discrepancy was due to CA being used as a standard for determination of TP in all the extracts in this work. As its molecular mass is 3.5-fold lower than that of verbascoside, the calculated results gave seemingly lower mass of TP. In various studies verbascoside demonstrated various pharmacological activities related to diabetes, including antioxidant and anti-inflammatory, as well as neuro- and hepato-protective activity. It also reduced glucose absorption rate through inhibition of both α-amylase and sodium-dependent glucose cotransporter 1 (SGLT1)-mediated glucose absorption in Caco-2 cells. In addition, verbascoside may protect β-cells against oxidative stress (34).

Another group of phenolics whose consumption may be connected to the prevention of T2D and other chronic diseases are flavonoids. Flavonoids may protect against high glucose levels by increasing insulin secretion and enhancing insulin sensitivity. They may also lower oxidative stress, prevent pancreatic β-cells from undergoing apoptosis, and reduce diabetes risk factors by regulating the major pathways for carbohydrate metabolism and hepatic glucose homeostasis (7). As for TF content (Table II), CI50 and CI10 were the extracts with their highest amount (61.09 ± 2.21 mg QE g^{-1} DM and 58.8 ± 2.87 mg QE g^{-1} DM, respectively), whereas MSy10 contained the least TF (9.98 ± 0.14 mg QE g^{-1} DM). In general, TF content constituted around 10 % of TP in most the extracts, a notable exception being CI extracts where they reached as much as 20 % share.

The results revealed that the extracts contained several common flavonoids such as apigenin, luteolin, and quercetin derivatives (Table III). Among them Q and its derivatives have received the most attention for their antidiabetic properties. Quercetin and consequently its derivatives may reduce hyperglycemia and liver glucose content. They may also improve glucose tolerance, hepatic glucokinase activity, release of insulin, and pancreatic cell regeneration, to name a few (7). While small amounts of free quercetin were present only in MSy extracts, the quantities of its glycosides were much higher. Most frequent among them was H present in AM, MS, and TPr extracts. Most abundant among them was MS50 with 7.738 mg H g^{-1} DM. Qcit, on the other hand, was present only in MS and MSy extracts. Again, MS50 was the richest in Qcit with as much as 8.677 mg Qcit g^{-1} DM. IQ was present only in TPr extracts with maximum of 2.340 mg g^{-1} DM in TPr50.

567 *Table III. Content of the selected phenolics in the extracts*

Extract	Caffeic acid derivatives (mg g^{-1} DM)	Apigenin derivatives (mg g^{-1} DM)	Luteolin derivatives (mg g^{-1} DM)	Quercetin derivatives (mg g^{-1} DM)
AM-10	0.205 (CA), 22.091 (ChA), 0.793 (NcA), 2.842 (RA)	0.440 (A), 0.124 (A7G)	0.561 (L), 3.625 (L7G)	2.278 (H)
AM-50	0.164 (CA), 23.332 (ChA), 0.683 (NcA), 7.722 (RA)	0.821 (A), 0.441 (A7G)	1.268 (L), 4.348 (L7G)	2.580 (H)
CI-10	12.103 (ChA)		0.692 (L7G)	
CI-50	15.054 (ChA)		0.794 (L7G)	
MS-10	2.087 (ChA)			3.572 (H), 3.717 (Qcit)
MS-50	3.359 (ChA)			7.738 (H), 8.677 (Qcit)
MSy-10	0.807 (ChA)	0.382 (A)		0.495 (Q), 0.637 (Qcit)
MSy-50	1.270 (ChA)	0.663 (A)		0.657 (Q)
PL-10	0.076 (CA), 2.187 (ChA), 1.430 (NcA), 329.800 (V)		0.219 (L)	
PL-50	0.085 (CA), 2.233 (ChA), 1.510 (NcA), 453.700 (V)		0.135 (L)	
TPr-10	0.154 (CA), 0.981 (ChA), 0.133 (NcA)			1.299 (H), 0.813 (IQ)

TPr-50	0.056 (CA), 1.382 (ChA), 0.270 (NcA)	0.606 (A7G)	4.272 (H), 2.340 (IQ)

568 CA – cafeic acid, ChA –chlorogenic acid, NcA – neochlorogenic acid, RA – rosmarinic acid, V – verbascoside, L – luteolin, L7G – luteolin-7-*O*-
569 glucoside, Q – quercetin as dehydrate, H – hyperosid, IQ – isoquercetin, Qcit – quercitrin as dehydrate, A – apigenin, A7G – apigenin-7-*O*-
570 glucoside, DM – dry mass of the extract. The extracts' names abbreviations are explained in the Experimental section “Preparation of the extracts”.
571

572 Apigenin and its derivatives also may have a significant
573 antidiabetic potential as it was shown that it may improve renal
574 dysfunction and oxidative stress, decrease blood glucose and
575 stimulate glucose induced insulin secretion (35). However,
576 apigenin and its derivatives were present in relatively small
577 amounts in the prepared extracts. Only AM and MSy extracts
578 contained A, the highest content being 0.821 mg/g DM in AM50.
579 Similarly, A7G was present in AM extracts and TPr50, the latter
580 containing the most of this glycoside (0.606 mg g^{-1} DM).
581 Content of luteolin derivatives in the extracts, on the other hand,
582 was somewhat higher (1.268 mg g^{-1} DM of L and 4.348 mg g^{-1}
583 DM of L7G, both in AM-50). Luteolin also shows an interesting
584 antidiabetic potential. Cumulative evidence indicates that
585 luteolin may be considered as a regulator of insulin resistance in
586 diabetes because it positively affects gluconeogenic and
587 lipogenic capacity (36).

588 It should be noted that 50 % ethanol was overall a better
589 solvent than 10 % ethanol for the extraction of all the
590 investigated phenolics as its use resulted in statistically higher
591 yields (paired *t*-test, $p < 0.05$) of TP, TCA, and TF. This is
592 probably due to the lower polarity of 50 % ethanol as compared
593 to 10 % ethanol, which was apparently more suitable for
594 dissolving the tested phenolic substances. The difference,
595 however, was not that high, as the ratios of the average contents
596 of selected phenolics obtained using 50 % ethanol and 10 %
597 ethanol ranged from 1.35 (TP) to 1.48 (TF). In general, the
598 results obtained in this work are in line with the results of some
599 previous studies that compared the content of phenols and
600 flavonoid in the investigated plants e.g. for AM (37). While the
601 presence of some of the selected phenolic standards confirms
602 previous reports (*e.g.* ChA in CI, PL, TPr, CA, hyperosid and
603 verbascoside in PL, the other previously reported compounds
604 were not recorded in this work (*e.g.* apigenin and A7O in PL)
605 (28). This may be related either to the interspecies variations (38)

or the specific conditions of extracts' preparation. This finding further stresses the well-known necessity of the standardization of herbal material used for preparation of plant-based products including those intended for T2D treatment (39).

Antioxidant activity of the extracts

Constant hyperglycemia, the hallmark of T2D, may contribute to the formation of advanced glycation end-products, the activation of protein kinase C, mitochondrial dysfunction, and ultimately the accumulation of ROS. Although ROS are essential for healthy metabolic processes at physiological levels, an overproduction of ROS can induce oxidative stress that further induces damage to cellular macromolecules, disruption of protein function, and, ultimately, cell death (4). A persistent condition of increased oxidative stress in T2D contributes to the onset of diabetic complications such as nephropathy, neuropathy, retinopathy, and liver damage (3, 4). Such state may be ameliorated by using various endogenous and exogenous antioxidants leading researchers to believe that the main benefit of herbal products in relation to T2D may be found primarily in the area of prevention of diabetic complications (40). The antioxidant properties of plant secondary metabolites can occur through different mechanisms, such as the transfer of hydrogen atoms or single electrons. The proportion of each of those mechanisms in total antioxidant activity of an herbal extract depends on various influences. Consequently, it is often rational to employ multiple methods to achieve a thorough evaluation of the antioxidant activity of complex mixtures, such as herbal extracts (41). In this work, antioxidant activity of the extracts was assessed and compared using RSA, ACLA, RP, and ORAC assay (Table II).

The data presented in Table II indicate moderate to relatively good antioxidant activity of the extracts in the RSA assay. Yet, none of the extracts reached the activity of the

antiradical standard, BHA (IC_{50} of 9.58 ± 0.38 μg g^{-1} DM). The best RSA (indicated by the lowest IC_{50} value) was displayed by CI50 and MS50 with IC_{50} values being 32.73 ± 2.22 μg g^{-1} DM and 26.94 ± 0.46 μg g^{-1} DM, respectively. The activity in this assay correlated statistically significantly with the TP and TCA content with $R^2 = 0.652$ ($p = 0.0015$) and $R^2 = 0.400$ ($p = 0.0273$) for TP and TCA, respectively. This indicates an important role that the phenolic compounds play in the radical scavenging activity of the extracts. Similar to the phenolic content, 50 % ethanol was found to be a better solvent for preparation of the extracts with high RSA, as its application resulted in the extracts displaying statistically lower IC_{50} values in this assay (paired *t*-test, $p < 0.05$). Unlike RSA, ACLA assay did not show any correlation with the content of the investigated phenolics, nor was the activity connected with the solvent used for the extraction. The most active in the ACLA assay was MS10 (IC_{50} = 31.54 ± 2.80 μg g^{-1} DM), but its activity did not quite reach that of the standard, BHA (IC_{50} = 2.03 ± 0.05 μg g^{-1} DM).

RP activity, on the other hand, was rather well pronounced in all the tested extracts. The most active extracts were CI50 ($EC_{0.5}$ = 0.13 ± 0.01 μg g^{-1} DM), MS50 ($EC_{0.5}$ = 0.12 ± 0.02 μg g^{-1} DM), and PL50 ($EC_{0.5}$ = 0.15 ± 0.01 μg g^{-1} DM), whose activity did not differ from the activity of the reducing standard, ascorbic acid ($EC_{0.5}$ = 0.12 ± 0.03). Similar to RSA assay, phenolic compounds contributed highly to the activity in RP assay, as the RP activity correlated significantly with their content ($R^2 = 0.7094$, $p = 0.0006$ for TP, $R^2 = 0.3707$, $p = 0.0356$ for TCA; and $R^2 = 0.3551$, $p = 0.0409$ for TF). This was not surprising having in mind that natural flavonoids and other phenols possess reducing abilities (42). The correlation between RSA and RP ($R^2 = 0.5957$, $p = 0.0033$), that was also observed, is probably due to similar underlying reasons. Finally, the extracts displayed excellent ORAC activity with TPr50 being by far the most active among the tested extracts (7354.71 ± 365.39

674 μmol TE g^{-1} DM). Similar to RSA and RP assays, 50 % ethanol
675 was better suited for the preparation of the extracts with high
676 ORAC activity than 10 % ethanol (paired *t*-test, $p < 0.05$).
677 Relatively good activity of several plants in the performed assays
678 has been previously recorded (43) but the overall comparison of
679 normalized activities showed that MS50, TPr50, and CI50
680 scored highest in the performed tests.
681
682 *Anti-inflammatory and collagenase-inhibitory activity*
683 One of the key features of T2D is chronic inflammation that
684 is observed in various tissues. It is directly involved in long-term
685 complications of diabetes, including cardiovascular disease,
686 nephropathy, retinopathy, and non-alcoholic fatty liver disease.
687 Furthermore, chronic inflammation in T2D is associated with
688 other conditions whose frequency is higher in patients suffering
689 from the disease, such as rheumatoid arthritis, gout or even
690 Alzheimer's disease (2). In this work, the anti-inflammatory
691 potential of the prepared extracts was investigated by using three
692 assays. The first was LOX-inhibition assay. LOX is the enzyme
693 involved in the metabolism of arachidonic acid and the release
694 of various pro-inflammatory eicosanoid substances, such as
695 leukotrienes and lipoxins. It mediates inflammatory events that
696 result from various environmental factors including sun
697 radiation, inflammation mediators, and allergens (44). Among
698 the extracts tested in this work, MS10 was the most potent LOX-
699 inhibitor with IC_{50} value being 45.03 ± 1.08 μg g^{-1} DM, which
700 was still weaker than the activity of NDGA (IC_{50} = 2.96 ± 0.33
701 μg g^{-1} DM) (Table IV). It seems that natural products with high
702 antioxidant capacity are important for LOX-inhibition activity as
703 the activity in this assay correlated statistically significant with
704 TP (R^2 = 0.4341, p = 0.0198), RSA (R^2 = 0.8558, p = 0.0004),
705 ACLA (R^2 = 0.5062, p = 0.0095) and RP (R^2 = 0.7056, p =
706 0.0006). Strong correlation of LOX-inhibition activity with RSA
707 and other antioxidant assays is in line with the redox mechanism

of LOX-inhibition activity, as LOX isoenzymes catalyze the stereospecific catalysis and oxygenation of polyunsaturated fatty acids to their hydroperoxy derivatives (45).

The second assay used in this work investigated the ability of the extracts to inhibit the heat-induced protein coagulation (Table IV). Denaturation of tissue proteins is one of the characteristics and causes of inflammatory processes. Therefore, its suppression may hinder the development of tissue changes which are another important aspect of inflammatory processes (22). The extracts displayed excellent activities in this assay, with several among them reaching the activity of the standard inhibitor, diclofenac sodium (IC_{50} = 114.66 ± 2.40 µg g^{-1} DM). The most active was MSy50 (IC_{50} = 80.09 ± 7.35 µg g^{-1} DM), while the others (CI10, CI50, MS50, Msy10) displayed statistically equal activity with values IC_{50} that ranged from 108.10 ± 3.07 µg g^{-1} DM to 137.15 ± 9.42 µg g^{-1} DM.

Collagenases belong to a larger group of matrix metalloproteinases (MMPs), enzymes that are involved in the development and healing of diabetic wounds. The management of wounds in diabetic patients presents significant challenges, as these wounds are linked to lower life quality and expectancy. One of the fundamental factors contributing to the chronic nature of diabetic wounds is the persistent state of inflammation occurring within the organism of diabetic patients. An excess of proinflammatory cytokines prompts fibroblasts to produce an overabundance of MMPs, which in turn degrade both nonviable and viable collagen fibers (46). Collagenase-inhibiting properties of the extracts are presented in Table IV. Most extracts displayed an excellent anti-collagenase activity with IC_{50} values that did not differ from that of the employed standard, gallic acid (IC_{50} = 127.82 ± 5.20 µg g^{-1} DM). The most active among them was TPr50 with IC_{50} = 73.58 ± 0.74 µg/g DM. Collagenase-inhibitory activity correlated significantly with TP (R^2 = 0.4341, p = 0.0198), designating phenolic

742 compounds as potentially significant contributors to this aspect
743 of the activity of the prepared extracts.

744 Among the tested extracts CI50, MS50, and CI10 showed
745 the best overall activity in the three performed assays, while
746 CI50, MSy50, and MS10 were the most active in the two assays
747 more closely related to the anti-inflammatory activity (LOXI and
748 OVA). Interestingly, a literature report states that the sprouts of
749 MS (47) did not contain lipoxygenase inhibitors. Thus, the
750 excellent activity demonstrated in this study is probably related
751 to the metabolites that develop in the later stages of plant growth.
752 While MSy was not, to the best of our knowledge, previously
753 investigated using the applied assays, previous studies have
754 shown that the hairy roots of CI were an excellent source of anti-
755 LOX compounds (48). However, to the best of our knowledge,
756 this is the first report on the activity of the aerial parts of CI for
757 anti-LOX activity. Interestingly, in a study investigating anti-
758 collagenase and anti-elastase activities of extracts from 21
759 plants, MS was found devoid of the activity (49), a result directly
760 opposing strong activity of the plant recorded in this work. While
761 CI (50), and MSy (51) have well-documented wound healing
762 activities, neither of them was specifically tested for anti-
763 collagenase activity. Thus, this work may be a constructive
764 addition to the research on their possible mechanism of action.

765 *Table IV. Antiinflammatory and antidiabetic activity of the extracts*

Extract	LOXI IC_{50} (μg g^{-1} DM)	OVA IC_{50} (μg g^{-1} DM)	COLL IC_{50} (μg g^{-1} DM)	aAM IC_{50} (μg g^{-1} DM)	aGL IC_{50} (μg g^{-1} DM)
AM10	$168.72 \pm 5.36^{d,x}$	$632.67 \pm 43.54^{c,d,x}$	$138.38 \pm 19.37^{b,c,y}$	*n.a.*	*n.a.*
AM50	$91.69 \pm 3.87^{h,x}$	$523.66 \pm 79.08^{d,x}$	$269.73 \pm 8.09^{b,c,y}$	$2100.64 \pm 217.90^{b,x}$	$828.33 \pm 32.96^{b,c,x}$
CI10	$171.44 \pm 13.79^{d,x}$	$137.15 \pm 9.42^{d,y}$	$109.25 \pm 1.09^{b,c,y}$	*n.a.*	*n.a.*
CI50	$114.79 \pm 7.82^{g,h,x}$	$108.10 \pm 3.07^{d,y}$	$84.24 \pm 11.79^{b,c,y}$	$2344.83 \pm 147.50^{b,x}$	$1122.62 \pm 61.78^{b,x}$
MS10	$45.03 \pm 1.08^{i,x}$	$1488.35 \pm 56.45^{a,x}$	$2883.77 \pm 346.05^{a,x}$	$204.10 \pm 2.11^{c,d,y}$	$11.29 \pm 0.07^{d,y}$
MS50	$146.26 \pm 7.05^{f,g,x}$	$116.01 \pm 6.24^{d,y}$	$102.12 \pm 15.32^{b,c,y}$	$78.27 \pm 0.99^{d,y}$	$3.21 \pm 0.04^{d,y}$
MSy10	$468.61 \pm 15.14^{a,x}$	$118.57 \pm 5.86^{d,y}$	$379.48 \pm 22.77^{b,x}$	*n.a.*	*n.a.*
MSy50	$355.17 \pm 18.09^{c,x}$	$80.09 \pm 7.35^{d,y}$	$197.89 \pm 27.70^{b,c,y}$	$4884.79 \pm 462.98^{a,x}$	$2584.71 \pm 328.60^{a,x}$
PL10	$191.43 \pm 8.91^{d,x}$	$362.55 \pm 36.00^{e,x}$	$106.95 \pm 8.56^{b,c,y}$	*n.a.*	*n.a.*
PL50	$156.45 \pm 12.18^{d,e,f,x}$	$840.08 \pm 116.63^{b,x}$	$110.94 \pm 8.88^{b,c,y}$	*n.a.*	$2314.91 \pm 461.05^{a,x}$
TPr10	$412.46 \pm 13.34^{b,x}$	$703.14 \pm 0.76^{b,x}$	$165.96 \pm 1.66^{b,c,y}$	$740.43 \pm 26.28^{c,x}$	$367.3 \pm 4.43^{c,d,y}$
TPr50	$149.75 \pm 19.88^{e,f,g,x}$	$693.52 \pm 60.23^{b,c,x}$	$73.58 \pm 0.74^{b,c,y}$	$552.22 \pm 14.10^{c,y}$	$240.83 \pm 9.75^{d,y}$
Standard	$^{(1)}$ 2.96 ± 0.33^{y}	$^{(2)}$ 114.66 ± 2.40^{y}	$^{(3)}$ 127.82 ± 5.20^{y}	$^{(4)}$ 284.74 ± 3.81^{y}	$^{(4)}$ 168.13 ± 0.46^{y}

DM – dry mass of the extract, LOXI – lipoxygenase inhibitory activity, OVA – activity in the ovalbumin coagulation assay, COLL – collagenase inhibitory activity, aAM – α-amylase inhibitory activity aGL – α-glucosidase inhibitory activity, *n.a.* – no activity. Standards: (1) nordihydroguaiaretic acid, (2) diclofenac sodium, (3) gallic acid, (4) acarbose.

[a-i] Differences between the extracts within a column (extracts not connected with the same capital letter are statistically different, Tukey post-test, $p < 0.05$); [x,y] differences with the positive control within a column (extracts not connected with the same capital letter are statistically different, Dunnet's post-test, $p < 0.05$). Values are average of 3 replications ± SD.

Inhibiting activity on α-amylase- and α-glucosidase

Antihyperglycaemic potential of the extracts was investigated by studying their potential to inhibit two enzymes involved into carbohydrate digestion: *α*-glucosidase and *α*-amylase. Numerous plant-derived secondary metabolites have the potential to influence critical enzymes involved in carbohydrate metabolism, thereby slowing the rise of postprandial glucose levels. The enzyme *α*-amylase is produced in the saliva and pancreatic juice. It facilitates the hydrolysis of starch into a mixture of oligosaccharides, which are subsequently broken down into glucose by *α*-glucosidase, the enzyme found in the mucosal brush border of the small intestine. Traditional medicinal plants are particularly abundant in *α*-amylase and *α*-glucosidase inhibitors, rendering them significant nutritional and therapeutic resources for the prevention of long-term complications associated with T2D, obesity, and hyperlipidemia (5, 52). The extracts investigated in this work displayed similar and rather notable inhibition of these two enzymes (Table IV).

While some extracts did not adversely affect the activity of the *α*-amylase in the respective assay, several extracts demonstrated high activity. Most active were the extracts prepared from the Fabaceae family, namely MS10, MS50, and TPr50. Their activity did not statistically differ from the activity of acarbose. Especially active was the MS50 whose IC_{50} value was 3.6-fold lower than then IC_{50} value of acarbose (78.27 ± 0.99 µg/g DM *vs.* 284.74 ± 3.81 µg/g DM). Similarly, the two extracts prepared from the same plants excelled as *α*-glucosidase-inhibitors. Superior activity was observed by MS10 (IC_{50} = 11.29 ± 0.07 µg/g DM), and MS50 (IC_{50} = 3.21 ± 0.04 µg/g DM), whose IC_{50} values were 15- and as much as 52-fold lower than IC_{50} value of acarbose. An association of α-glucosidase-inhibiting assay with the activity in the ORAC assay was found

but it was rather low (R^2 = 0.5318, p = 0.0401). However, the activity in α-glucosidase-inhibiting assay was rather strongly correlated with α-amylase-inhibiting activity (R^2 = 0.8269, p = 0.0045) indicating either the similar underlying mechanisms of the two assays or the presence of chemically similar inhibitors in the tested extracts.

Interestingly, while in this study MSy extracts displayed either weak or no discernible activity, a in a study performed on samples from Palestine, hydrophilic fractions of the plant exhibited a remarkable α-amylase inhibitory activity (53). The discrepancy could be attributed to differences in chemical composition that occur in different geographical regions as it has been noted that chemical composition and biological activity of MSy is strongly influenced by altitude, fertility of soil, and water supply, even when growing in close geographic areas (38). Overall, the best combined inhibition was observed by MS50, MS10, and TPr50 extract. Previous studies of MS (43), CI, and TPr extracts (8) demonstrated moderate antidiabetic activity in the two assays. However, the excellent activity presented in this work is, in addition to the chemical interspecies diversity, possibly a consequence of the selected solvent and/or the extraction method (*e.g.* ultrasonication *vs.* accelerated solvent extraction and laser irradiation that were used in ref. 43).

CONCLUSIONS

The results indicate that, due to the presence of active substances which could alleviate the negative effects of diabetes, the examined biowaste material from urban parks could be of importance for the pharmaceutical industry in preparation of high-value antidiabetic products. In general, ethanol in concentration of 50 % was shown to be better suited than 10 % ethanol for extraction of active principles of the selected plants emphasizing the important role that the solvent selection plays

in the development of plant extracts-based products. Among the tested extracts, those prepared from CI, MS, and TPr repeatedly demonstrated superior antidiabetic potential due to their high phenolic content, strong antioxidant, and anti-inflammatory activity, as well as strong inhibition of α-glucosidase and α-amylase. Future work should focus on pinpointing their active principles, a task needed for potential preparation and standardization of the antidiabetic medicines and/or food supplements based on these plants.

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